

Claims

1. A DNA construct comprising a transcriptional unit which comprises a ribonucleotide reductase gene and a thioredoxin gene or a uridine kinase gene and/or a dCTP deaminase gene.
2. A DNA construct as claimed in claim 1 comprising a transcriptional unit which comprises a ribonucleotide reductase gene and a thioredoxin gene.
3. A DNA construct as claimed in claim 1 comprising a transcriptional unit which comprises a uridine kinase gene and/or a dCTP deaminase gene.
4. A DNA construct as claimed in claim 1 comprising a transcriptional unit which comprises a uridine kinase gene and a dCTP deaminase gene.
5. A DNA construct according to any of claims 1-4 wherein the construct is an extrachromosomal vector.
6. A DNA construct according to claim 5 wherein the vector is a plasmid, virus, transposon, minichromosome or phage.
7. A DNA construct according to claim 6 wherein the vector is a plasmid, wherein the ribonucleotide reductase gene is a *nrdA* gene and/or a *nrdB* gene, and wherein the thioredoxin gene is a *nrdC* gene and said genes are arranged in an operon.
8. A DNA construct according to claim 7 wherein the transcriptional unit comprises a *nrdA* gene and a *nrdB* gene.
9. A DNA construct according to claim 8 in which the *nrdA* and *nrdB* genes are located downstream of the *nrdC* gene.
10. A DNA construct according to claim 8 wherein the *nrdC* gene is upstream of the *nrdA* gene and *nrdA* gene is upstream of the *nrdB* gene.

11. A DNA construct according to claim 6, further comprising a regulatory element.
12. A DNA construct according to claim 11, wherein the regulatory element is selected
5 from the group consisting of a promoter, an operator, a termination sequence, an initiation sequence and a ribosome binding site.
13. A DNA construct according to claim 12 wherein the promoter is the lambda P_L promoter or a derivative therefrom.
- 10 14. A DNA construct according to claim 12 wherein the termination sequence is a synthetic terminator.
- 15 15. A DNA construct according to claim 7 wherein the *nrdA* gene is modified such that ribonucleotide reductase encoded by the unit is less sensitive to allosteric inhibition than the ribonucleotide reductase encoded by the unit comprising an unmodified *nrdA* gene.
- 20 16. A DNA construct according to claim 15, wherein the *nrdA* gene is modified at a dTTP binding site.
17. A DNA construct according to claim 15 wherein the *nrdA* gene is modified so as to encode an Ala-to-Ile change at position 79 in the *nrdA* expression product.
- 25 18. A DNA construct according to claim 7 wherein the *nrdA*, *nrdB* and *nrdC* genes are derived from a T phage.
19. A DNA construct according to claim 18 wherein the T phage is a T even phage.
- 30 20. A DNA construct according to claim 19 wherein the T even phage is a T4 phage.
21. A DNA construct according to any of claims 1-4 wherein the construct further comprises a thymidylate synthase gene.

22. A DNA construct according to claim 21 wherein the thymidylate synthase gene is the *td* gene.

5 23. A DNA construct according to claim 22 wherein *td* gene is located on the same vector as the *nrdA*, *nrdB* and *nrdC* genes.

24. A DNA construct according to claim 22 wherein the *td* gene is located in the same operon as the *nrdA*, *nrdB* and *nrdC* genes.

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25. A DNA construct according to claim 24 wherein the *td* gene is located downstream (in terms of reading frame) from the *nrdA*, *nrdB* and *nrdC* genes.

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26. A DNA construct according to claim 22 wherein the *td* gene is derived from a T phage.

27. A DNA construct according to claim 26 wherein the *td* gene is derived from a T even phage.

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28. A DNA construct according to claim 27 wherein the *td* gene is derived from T4 phage.

29. A DNA construct according to claim 2 further comprising a uridine kinase gene and/or a dCTP deaminase gene.

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30. A DNA construct according to claim 29, wherein the DNA construct comprises both a uridine kinase gene and a dCTP deaminase gene.

31. A DNA construct according to claim 1,3 or 4 wherein the uridine kinase gene is a *udk* gene.

32. A DNA construct according to claim 1,3 or 4 wherein the dCTP deaminase gene is a *dcd* gene

33. A modified host cell comprising a DNA construct according to any of the preceding claims.

5 34. A modified host cell according to claim 33 wherein the host cell displays repressed or no uracil DNA glycosylase activity.

35. A modified host cell according to claim 34 wherein one or more host cell DNA sequences encoding for uracil DNA glycosylase activity have been modified so as
10 to encode expression products displaying repressed, low levels of or no uracil DNA glycosylase activity.

36. A modified host cell according to claim 35 wherein the host cell DNA sequence is an *ung* gene.

15 37. A modified host cell according to claim 34 wherein one or more host cell DNA sequences encoding for uracil DNA glycosylase activity have been removed.

38. A modified host cell according to claim 33 wherein the cell is a eukaryote or a
20 prokaryote.

39. A modified host cell according to claim 38 wherein the host cell is selected from the group consisting of *E. coli*, *Salmonella*, *Pseudomonas*, *Bacillus* and *Saccharomyces*.

25 40. A modified host cell comprising a DNA construct, which construct comprises a transcription DNA unit, which unit comprises a ribonucleotide reductase gene and a thioredoxin gene, wherein said ribonucleotide reductase displays less sensitivity to allosteric inhibition than the wild-type equivalent of the reductase, and wherein
30 said host cell further comprises one or more of the following features:

- (a) a transcription unit located on said DNA construct, comprising a thymidylate synthase gene heterologous to the thymidylate synthase gene of the host cell;

- (b) a transcription unit located on said DNA construct, comprising a uridine kinase gene;
- (c) a transcription unit located on said DNA construct, comprising a dCTP deaminase gene; and
- 5 (d) repressed or absent uracil DNA glycosylase activity.

41. A modified host cell according to claim 40, wherein the ribonucleotide reductase gene is modified at a dTTP binding site.

10 42. A modified host cell according to claim 41, wherein the ribonucleotide reductase is the *nrdA* gene and the modification is an Ala-to-Ile change at position 79 in the *nrdA* expression product.

15 43. A modified host cell according to claim 38, wherein the DNA construct comprises both the uridine kinase gene and the dCTP deaminase gene.

44. A modified host cell according to claim 40, wherein the cell comprises each one of the features of (a) to (d).

20 ~~45. A process for the production of pyrimidine deoxyribonucleosides comprising culturing a host cell according to any of claims 33-44.~~

46. A process according to claim 45 wherein the deoxyribonucleoside is thymidine.

25 ~~47. A process for the manufacture of azidothymidine comprising culturing a host cell according to any of claims 33-44.~~

30 ~~48. A culture medium comprising a host cell according to any of claims 33-44.~~